PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 4 : C07K 15/00, C12P 21/00 G01N 33/577, 33/569, A61K 39/40 // C12N 15/00 (C12P 21/00 C12R 1:91)

A1

(11) International Publication Number: **WO 86/ 02355**

(43) International Publication Date: 24 April 1986 (24.04.86)

(21) International Application Number: PCT/GB85/00466

(22) International Filing Date: 16 October 1985 (16.10.85)

(31) Priority Application Number: 8426468

(32) Priority Date: 19 October 1984 (19.10.84)

(33) Priority Country: GB

(71) Applicant *(for all designated States except US)*: TECHNOLOGY LICENCE COMPANY LIMITED [GB/GB]; 24 Finch Road, Douglas, Isle of Man (GB).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)* : WRIGHT, Bruce, William [GB/GB]; 48 Bush Close, Comberton, Cambridgeshire CB3 7EG (GB). COX, Peter, John [GB/GB]; Church Cottage, Church Road, Chevington, Bury St. Edmunds, Suffolk IP19 5QH (GB). NOYES, Alice, Margaret [GB/GB]; 68 High Street, Willingham, Cambridgeshire CB4 5ES (GB). WIDDOWS, Danny [GB/GB]; 14 Sherbourne Close, Cambridge CB4 1RT (GB). COPLEY, Clive, Graham [GB/GB]; Basement Flat, 84 Milton Road, Cambridge CB4 1LA (GB).

(74) Agent: GILL JENNINGS & EVERY; 53/64 Chancery Lane, London WC2A 1HN (GB).

(81) Designated States: AT (European patent), BE (European patent), CH (European patent), DE (European patent), FR (European patent), GB (European patent), IT (European patent), JP, LU (European patent), NL (European patent), SE (European patent), US.

Published
With international search report.
Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: MONOCLONAL ANTIBODIES AND THEIR USE

(57) Abstract

Monoclonal antibodies to the genus *Chlamydia*, the labelled antibodies, compositions and kits containing them, and their use in diagnosis of antigen and treatment.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AT	Austria	GA	Gabon	MR	Mauritania
AU	Australia	GB	United Kingdom	MW	Malawi
BB	Barbados	HU	Hungary	NL	Netherlands
BE	Belgium	IT	Italy	NO	Norway
BG	Bulgaria	JP	Japan	RO	Romania
BR	Brazil	KP	Democratic People's Republic of Korea	SD	Sudan
CF	Central African Republic			SE	Sweden
CG	Congo	KR	Republic of Korea	SN	Senegal
CH	Switzerland	LI	Liechtenstein	SU	Soviet Union
CM	Cameroon	LK	Sri Lanka	TD	Chad
DE	Germany, Federal Republic of	LU	Luxembourg	TG	Togo
DK	Denmark	MC	Monaco	US	United States of America
FI	Finland	MG	Madagascar		
FR	France	ML	Mali		

MONOCLONAL ANTIBODIES AND THEIR USE

BACKGROUND OF THE INVENTION

Of current interest in the fields of analysis and diagnosis is the use of monoclonal antibodies to determine the presence of antigens or species in specimens such as urine, blood, water, milk, and the like.

More particularly, monoclonal antibodies specific for the antigens or species of Chlamydia are desired which when used will rapidly diagnose the presence of such organisms in specimens.

Divisions have been made among the Chlamydia species. Some of the representative members include Chlamydia trachomatis, and Chlamydia psittaci.

The Chlamydiae are obligate intracellular parasites of birds and mammals. Four human diseases are caused by Chlamydiae: psittacosis, lymphogranuloma venereum; and two diseases of the eye, trachoma and inclusion conjunctivitis. Psittacosis is caused by C. psittaci, while the latter three diseases are usually caused by C. trachomatis.

Chlamydia is, in many areas, the most common cause of venereal disease. This subtle, often apparently mild disease, is now recognized as a frequent cause of deep infections in the genital

tracts of males and females, probably the leading cause of female infertility and male strictures. Diagnosis is often imperfect. Present methods include smears which are non-specific, and cultures which are slow and costly. The ability of monoclonal antibodies specifically to bind to antigens of Chlamydia can provide many opportunities for diagnosis and treatment. Such specificity is a most important requirement for proper and accurate analysis and/or diagnosis, particularly in diagnosing the presence of diseases which require prompt treatment.

A wide variety of isotopic and nonisotopic immunoassays have been utilized in conjunction with monoclonal antibodies to test for the presence of an antigenic substance. At the present time, agglutination, immuno-fluorescent, chemiluminescent or fluorescent immunoassay, immuno-electron microscopy, radiometric assay systems, radio immunoassays, and enzyme-linked immunoassays are the most common techniques used with the monoclonal antibodies. Other techniques include bioluminescent, fluorescence polarization, and photon-counting immunoassays.

When utilizing the enzyme-linked immunoassay procedure (EIA), it is necessary to bind, or

conjugate, the monoclonal antibody with an enzyme capable of functioning in such assay; such as alkaline phosphatase.

The enzyme-linked monoclonal antibody can then be used in the known enzyme-linked immunosorbent assay procedure to determine the presence of an antigenic substance.

After the specific antigen is identified, the serotype of the infecting organism can be determined, and appropriate treatment can then be initiated to rapidly and efficiently eliminate the disease.

The production of monoclonal antibodies is now a well-known procedure first described by Kohler and Milstein (Eur. J. Immunol. 6, 292 (1975)). While the general technique of preparing hybridomas and the resultant monoclonal antibodies is understood, it has been found that preparing a specific monoclonal antibody to a specific antigen is difficult, mainly due to the degree of specificity and variations required in producing a particular hybridoma.

SUMMARY OF THE INVENTION

The present invention provides novel monoclonal antibodies for use in accurately and rapidly diagnosing samples for the presence

of Chlamydia antigens and/or organisms.

Briefly stated, the present invention comprises monoclonal antibodies specific for an antigen or species of Chlamydia; in particular, the antigens or species of Chlamydia trachomatis (designated as C. trachomatis I, II, III, or IV), and the antigens or species of Chlamydia psittaci, as well as a monoclonal antibody broadly cross-reactive with an antigen for each species of the genus Chlamydia.

The invention also comprises labeled monoclonal antibodies for use in diagnosing the presence of the Chlamydia antigens, each comprising a monoclonal antibody against one of the above-mentioned antigens to Chlamydia or to a particular species thereof and linked thereto an appropriate label. The label can be chosen from the group consisting of a radioactive isotope, enzyme, fluorescent compound, chemiluminescent compound, bioluminescent compound, ferromagnetic atom, or particle, or any other label.

The invention further comprises the process for diagnosing the presence of Chlamydia antigens or organisms in a specimen comprising contacting said specimen with the labeled monoclonal antibody in an appropriate immunoassay procedure.

Additionally, the invention is also directed to a therapeutic composition comprising a monoclonal antibody for an antigen of Chlamydia and a carrier or diluent, as well as kits containing at least one labeled monoclonal antibody to an antigen of a Chlamydia.

DETAILED DESCRIPTION

The monoclonal antibodies of the present invention are prepared by fusing spleen cells, from a mammal which has been immunized against the particular Chlamydia antigen, with an appropriate myeloma cell line, preferably NS0 (uncloned), P3NS1-Ag4/1, or Sp2/0 Ag14. The resultant product is then cultured in a standard HAT (hypoxanthine, aminopterin, and thymidine) medium. Screening tests for the specific monoclonal antibodies are employed utilizing immunoassay techniques which will be described below.

The immunized spleen cells may be derived from any mammal, such as primates, humans, rodents (i.e., mice, rats, and rabbits), bovine, ovine, canine, or the like, but the present invention will be described in connection with mice. The mouse is first immunized by injection of the particular Chlamydia antigen chosen generally for a period of approximately eleven weeks.

When the mouse shows sufficient antibody production against the antigen, as determined by conventional assay, it is given a booster injection of the appropriate Chlamydia antigen, and then killed so that the immunized spleen may be removed. The fusion can then be carried out utilizing immunized spleen cells and an appropriate myeloma cell line.

The fused cells yielding an antibody which give a positive response to the presence of the particular Chlamydia antigen are removed and cloned utilizing any of the standard methods. The monoclonal antibodies from the clones are then tested against standard antigens to determine their specificity for the particular Chlamydia antigen. The monoclonal antibody selected, which is specific for the particular Chlamydia antigen or species, is then bound to an appropriate label.

Amounts of antibody sufficient for labeling and subsequent commercial production are produced by the known techniques, such as by batch or continuous tissue culture or culture in vivo in mammals, such as mice.

The monoclonal antibodies may be labeled with a multitude of different labels, such as

enzymes, fluorescent compounds, luminescent compounds, radioactive compounds, ferromagnetic labels, and the like. The present invention will be described with reference to the use of an enzyme labeled monoclonal antibody. Some of the enzymes utilized as labels are alkaline phosphatase, glucose oxidase, galactosidase, peroxidase, or urease, and the like.

Such linkage with enzymes can be accomplished by any one of the conventional and known methods, such as the Staphylococcal Protein A method, the glutaraldehyde method, the benzoquinone method, or the periodate method.

Once the labeled monoclonal antibody is formed, testing is carried out employing one of a wide variety of conventional immunoassay methods. The particular method chosen will vary according to the monoclonal antibody and the label chosen. At the present time, enzyme immunoassays are preferred due to their low cost, reagent stability, safety, sensitivity, and ease of procedure. One example is enzyme-linked immunosorbent assay (EIA). EIA is a solid phase assay system which is similar in design to the radiometric assay, but which utilizes an enzyme in place of a radioactive isotope

as the immunoglobulin marker.

Fluorescent-immunoassay is based on the labeling of antigen or antibody with fluorescent probes. A nonlabeled antigen and a specific antibody are combined with identical fluorescently labeled antigen. Both labeled and unlabeled antigen compete for antibody binding sites. The amount of labeled antigen bound to the antibody is dependent upon, and therefore a measurement of, the concentration of nonlabeled antigen. Examples of this particular type of fluorescent-immunoassay would include heterogenous systems such as Enzyme-Linked Fluorescent Immunoassay, or homogeneous systems such as the Substrate Labeled Fluorescent Immunoassay. The most suitable fluorescent probe, and the one most widely used is fluorescein. While fluorescein can be subject to considerable interference from scattering, sensitivity can be increased by the use of a fluorometer optimized for the probe utilized in the particular assay and in which the effect of scattering can be minimized.

In fluorescence polarization, a labeled sample is excited with polarized light and the degree of polarization of the emitted light is measured. As the antigen binds to the antibody

its rotation slows down and the degree of polarization increases. Fluorescence polarization is simple, quick, and precise. However, at the present time its sensitivity is limited to the micromole per liter range and upper nanomole per liter range with respect to antigens in biological samples.

Luminescence is the emission of light by an atom or molecule as an electron is transferred to the ground state from a higher energy state. In both chemiluminescent and bioluminescent reactions, the free energy of a chemical reaction provides the energy required to produce an intermediate reaction or product in an electronically excited state. Subsequent decay back to the ground state is accompanied by emission of light. Bioluminescence is the name given to a special form of chemiluminescence found in biological systems, in which a catalytic protein or enzyme, such as luciferase, increases the efficiency of the luminescent reaction. The best known chemiluminescent substance is luminol.

A further aspect of the present invention is a therapeutic composition comprising one or more of the monoclonal antibodies to the particular Chlamydia antigen or species, as

well as a pharmacologically acceptable carrier or diluent. Such compositions can be used to treat humans and/or animals afflicted with some form of Chlamydia infections and they are used in amounts effective to cure; an amount which will vary widely dependent upon the individual being treated and the severity of the infection.

One or more of the monoclonal antibodies can be assembled into a diagnostic kit for use in diagnosing for the presence of an antigen, antigens, or species of Chlamydia in various specimens. It is also possible to use the broadly cross-reactive monoclonal antibody which can identify the genus Chlamydia alone or as part of a kit containing antibodies that can identify other bacterial genera or species of Chlamydia and/or other bacteria.

In the past there have been difficulties in developing rapid kits because of undesirable cross-reactions of specimens with antiserum. The use of monoclonal antibodies can eliminate these problems and provide highly specific and rapid tests for diagnosis. A rapid and precise kit could replace or augment existing tests and permit early direct therapy using precise

antibiotics. Avoiding multiple antibiotics or more expensive or hazardous antibiotics would represent substantial patient and hospital savings. Additionally, a kit can be used on an out-patient basis. At present the lack of a rapid test giving "same day" answers may delay the initiation of treatment until the patient has developed more severe symptoms or may require the initiation of more costly therapy in a sick patient. A test that would return results within an hour or two would be a substantial convenience to patients.

In addition to being sold individually, the kit could be included as a component in a comprehensive line of compatible immunoassay reagents sold to reference laboratories to detect the species and serotypes of Chlamydia.

One preferred embodiment of the present invention is a diagnostic kit comprising at least one labeled monoclonal antibody against a particular Chlamydia antigen or species, as well as any appropriate stains, counterstains, or reagents. Further embodiments include kits containing at least one control sample of a Chlamydia antigen and/or a cross-reactive labeled monoclonal antibody which would detect the pres-

ence of any of the Chlamydia organisms in a particular sample. Specific antigens to be detected in this kit include the antigens of Chlamydia trachomatis (applicant has further divided this species into four subgroups: Chlamydia trachomatis I, II, III, or IV), and Chlamydia psittaci.

Monoclonal diagnostics which detect the presence of Chlamydia antigens can also be used in periodic testing of water sources, food supplies and food processing operations. Thus, while the present invention describes the use of the labeled monoclonal antibodies to determine the presence of a standard antigen, the invention can have many applications in diagnosing the presence of antigens by determining whether specimens such as urine, blood, stool, water, milk, and the like contain the particular Chlamydia antigen. More particularly, the invention could be utilized as a public health and safety diagnostic aid, whereby specimens such as water or food could be tested for possible contamination.

The invention will be further illustrated in connection with the following examples which are set forth for purposes of illustration only

and not by way of limitation.

In the Examples:

API = Analytical Profile Index (ref. Ayerst Labs)

DMEM = Dulbecco's Modified Eagles Medium

FCS = Foetal Calf Serum

PBS = phosphate-buffered saline

% T refers to vaccine concentration measured in a 1 cm light path

Monoclonal antibodies of the present invention are prepared generally according to the method of Koehler and Milstein, Eur. J. Immunol. 6, (1975) 292.

EXAMPLE 1

A. Antigen Preparation

Chlamydia trachomatis serotype LG2 is obtained from Dr. G. L. Ridgeway at the University College Hostpital. The antigen is grown by standard methods in cell culture.

B. Animal Immunisation

Balb/c mice are injected with the prepared antigen. They are given intraperitoneal and/or intravenous injections (0.05 ml 80% T vaccine) of vaccine prepared as above. The mice are bled approximately six days after the last injection and the serum tested for antibodies by assay. A conventional assay used for this serum titer testing is the enzyme-linked immunosorbent assay system. When the mice show antibody production after this regimen, generally a positive titer of at least 10,000, a mouse is selected as a fusion donor and given a booster injection (0.02 ml 80% T vaccine) intravenously, three days prior to splenectomy.

C. Cell Fusion

Spleen cells from the immune mice are harvested three days after boosting, by conventional techniques. First, the donor mouse selected is killed and surface-sterilised by immersion in 70% ethyl alcohol. The spleen is then removed and immersed in approximately

2.5 ml DMEM to which has been added 3% FCS. The spleen is then gently homogenised in a LUX homogenising tube until all cells have been released from the membrane, and the cells are washed in 5 ml 3% FCS-DMEM. The cellular debris is then allowed to settle and the spleen cell suspension placed in a 10 ml centrifuge tube. The debris is then rewashed in 5 ml 3% FCS-DMEM. 50 ml suspension are then made in 3% FCS-DMEM.

The myeloma cell line used is NSO (uncloned), obtained from the MRC Laboratory of Molecular Biology in Cambridge, England. The myeloma cells are in the log growth phase, and rapidly dividing. Each cell line is washed using, as tissue culture medium, DMEM containing 3% FCS.

The spleen cells are then spun down at the same time that a relevant volume of myeloma cells are spun down (room temperature for 7 minutes at 600 g), and each resultant pellet is then separately resuspended in 10 ml 3% FCS-DMEM. In order to count the myeloma cells, 0.1 ml of the suspension is diluted to 1 ml and a haemacytometer with phase microscope is used. In order to count the spleen cells, 0.1 ml of the suspension is diluted to 1 ml with Methyl Violet-citric acid solution, and a haemacytometer and light microscope are used to count the stained nuclei of the cells.

1×10^8 Spleen cells are then mixed with 6×10^7 myeloma cells, the mixture washed in serum-free DMEM high in glucose, and centrifuged, and all the liquid removed. The resultant cell pellet is placed in a 37°C water-bath. 1 ml of a 50 w/v solution of polyethylene glycol 1500 (PEG) in saline Hepes, pH approximately 7.5, is added, and the mixture gently stirred for approximately 1.5 minutes. 10 ml serum-free tissue culture medium DMEM are then slowly added, followed by up to 50 ml of such culture medium, centrifugation and removal of all the

supernatant, and resuspension of the cell pellet in 10 ml of DMEM containing 18% by weight FCS.

10 µl of the mixture are placed in each of 480 wells of standard multiwell tissue culture plates. Each well contains 1.0 ml of the standard HAT medium (hypoxanthine, aminopterin and thymidine) and a feeder layer of Balb/c macrophages at a concentration of 5×10^4 macrophages/well.

The wells are kept undisturbed, and cultured at 37°C in 9% CO_2 air at approximately 100% humidity. The wells are analysed for growth, utilising the conventional inverted microscope procedure, after about 5 to 10 days. In those wells in which growth is present in the inhibiting HAT medium, screening tests for the specific monoclonal antibody are made utilising the conventional enzyme immunoassay screening method described below. Somewhere around 10 days to 14 days after fusion, sufficient antibody against the antigen may develop in at least one well.

D. Cloning

From those wells which yielded antibody against the antigen, cells are removed and cloned using the dilution method. In limiting dilution, dilutions of cell suspensions in 18% FCS-DMEM + Balb/c mouse macrophages were made to achieve 1 cell/well and half cell/well in a 96-well microtitre plate. The plates were incubated for 7-14 days at 37 C, 95% RH, 7-9% CO_2 until semi-confluent. The supernatants were then assayed for specific antibody by the standard enzyme immunosorbent assay.

The clones may be assayed by the enzyme immunoassay method to determine antibody production.

E. Monoclonal Selection

The monoclonal antibodies from the clones are screened by the standard techniques for binding to the antigen, prepared as in the immunisation, and for

specificity in a test battery of the class bearing different antigens. Specifically, a grid of microtiter plates containing a representative selective of organisms is prepared, boiled, and utilised as a template to define the specificity of the parent group. The EIA immunoassay noted above may be used.

F. Antibody Production and Purification (2 alternatives)

(1) Six Balb/c mice are primed with pristane and injected intraperitoneally with 10^7 cells of the monoclonal antibody specific against the antigen. The ascites fluid is harvested after the mice have reached the proper stage; the mice are swollen with fluid but still alive.

The cells are then centrifuged at 1200 g for approximately 10 minutes, the cells discarded, and the antibody-rich ascites fluid collected. The fluid is titrated, as noted above, to establish presence and level of antibody, and purified.

Purification is accomplished using the protein A - Sepharose method. More particularly, about 10 ml of the ascites fluid are filtered through glass wool and centrifuged at 30,000 g for 10 minutes. The ascites is then diluted with twice its own volume of cold phosphate buffer (0.1 M sodium phosphate, pH 8.2). The diluted ascites is loaded on to a 2 ml column of protein A - Sepharose which has previously been equilibrated with phosphate buffer. The column is washed with 40 ml phosphate buffer, and the monoclonal antibody is eluted with citrate buffer (0.1 M sodium citrate, pH 3.5) into sufficient 1M tris buffer, pH 9.0, to raise the pH immediately to about 7.5. The eluate is dialysed in 2 x 1000 ml PBS at +4°C.

(2) Cells of the monoclonal antibody-producing line specific to Chlamydia trachomatis are grown in batch tissue culture. DMEM, to which has been added 10% FCS,

is used to support growth in mid-log phase, to 1 litre volume. The culture is allowed to overgrow, to allow maximum antibody production. The culture is then centrifuged at 1200 g for approximately 10 minutes. The cell/cell debris is discarded and the antibody-rich supernatant collected.

The fluid may then be titrated, as noted above, to establish presence and level of antibody, and purified by a combination of batch ion-exchange chromatography, ammonium sulphate precipitation and column ion-exchange (a possible alternative would be protein A - Sepharose) chromatography.

More particularly, to one litre of culture supernatant is added one litre of 0.05M sodium acetate buffer, pH 4.5, and 40 ml of SP-Sephadex, previously equilibrated in 0.1M sodium acetate buffer, pH 5.0. The suspension is stirred at +4°C for one hour. The SP-Sephadex is allowed to settle and the supernatant is decanted. The SP-Sephadex is packed in a column, washed with 60 ml of 0.1M acetate buffer, pH 5.0, and eluted with 60 ml of the same buffer plus 1M sodium chloride. The eluate is stirred at +4°C, and an equal volume of saturated ammonium sulphate added slowly. The suspension is stirred for a further 30 minutes. The precipitate is then harvested by centrifugation at 10,000 g for 10 minutes. The precipitate is dissolved in a minimum volume of either cold phosphate/EDTA buffer (20mM sodium phosphate, 10mM EDTA, pH 7.5, + 0.02% sodium azide) for DEAE-cellulose chromatography, or phosphate buffer (0.1M sodium phosphate, pH 8.2 + 0.02% sodium azide) for protein A-Sepharose chromatography. The dissolved precipitate is dialysed versus 2 x 1000 ml of the dissolution buffer at +4°C, and the appropriate chromatography step carried out as previously described.

G. <u>Enzyme-Monoclonal Linkage</u>

The monoclonal antibody specific against the antigen, prepared as above, is linked to an enzyme, viz. highly-purified alkaline phosphatase. The one-step glutaraldehyde method or benzoquinone conjugation is used.

In the one-step glutaraldehyde method, 3 mg monoclonal antibody (in about 1 ml of solution) are dialysed with 10 mg alkaline phosphatase (Sigma Type VII-T) against 2 x 1000 ml of PBS, pH 7.4, at +4°C. After dialysis, the volume is made up to 2.5 ml with PBS, and 25 µl of a 20% glutaraldehyde in PBS solution are added. The conjugation mixture is left at room temperature for 1.5 hours. After this time, glutaraldehyde is removed by gel filtration on a Pharmacia PH-10 (Sephadex G-25 M) column, previously equilibrated in PBS. The conjugate is eluted with 3.5 ml PBS and then dialysed against 2 x 2000 ml of TRIS buffer (50 mM TRIS, 1 mM magnesium chloride, pH 8.0, plus 0.02% sodium azide) at +4°C. To the dialysed conjugate is added 1/10th its own volume of 10% BSA in TRIS buffer. The conjugate is then sterile-filtered through a 0.22 µm membrane filter into a sterile amber vial and stored at +4°C.

EXAMPLE 2

The procedure of Example 1 was followed, but using Chlamydia trachomatis elementary bodies as the antigen, obtained from the London School of Hygiene and Tropical Medicine (strain title Chlamydia). The antigen was prepared by infecting the organisms into McCoy cells grown in microcarrier beads; the supernatant was removed and centrifuged first at low speed (c. 1000 rpm) and then at 20,000 g for 1 hour. The pellet was resuspended through renografin, frozen and thawed once.

Immunisation comprised a intramuscular injection (in Complete Freunds Adjuvant), followed 3 weeks later by an

ip injection (in PBS), followed 3 weeks later by an iv injection (in PBS).

EXAMPLE 3

The general procedure of Example 1 may be followed to produce a monoclonal antibody broadly cross-reactive with an antigen of all species of the genus Chlamydia.

Tests using the present invention are superior to existing tests, based on the following advantages: (i) greater accuracy; (ii) same day results, within an hour or two; (iii) reduction in amount of skilled labour required to administer laboratory procedures, resulting in reduced labour costs; (iv) reduction in laboratory time and space used in connection with tests, resulting in reduced overhead expenses; and (v) improved therapy based upon early, precise diagnosis.

While the invention has been described in connection with certain preferred embodiments, it is not intended to limit the scope of the invention to the particular form set forth but, on the contrary, it is intended to cover such alternatives, modifications, and equivalents as may be included within the spirit and scope of the invention as defined by the appended claims.

WHAT IS CLAIMED IS:

1. A monoclonal antibody specific for an antigen or species of Chlamydia.

2. The antibody of Claim 1 specific to the antigen or antigens of Chlamydia trachomatis.

3. The antibody of Claim 1 specific to the antigen or antigens of Chlamydia trachomatis I.

4. The antibody of Claim 1 specific to the antigen or antigens of Chlamydia trachomatis II.

5. The antibody of Claim 1 specific to the antigen or antigens of Chlamydia trachomatis III.

6. The antibody of Claim 1 specific to the antigen or antigens of Chlamydia trachomatis IV.

7. The antibody of Claim 1 specific to the antigen or antigens of Chlamydia psittaci.

8. A monoclonal antibody broadly cross-reactive with an antigen of all species of the genus Chlamydia.

9. A labeled monoclonal antibody consisting essentially of a monoclonal antibody of Claims 1-8 and an appropriate label.

10. The labeled monoclonal antibody of Claim 9, wherein said label is a member of the group selected from a radioactive isotope, enzyme, fluorescent compound, bioluminescent compound, chemiluminescent compound, or ferromagnetic atom, or particle.

11. The labeled monoclonal antibody of Claim 10, wherein said label is an enzyme capable of conjugating with a monoclonal antibody and of being used in an enzyme-linked immunoassay procedure.

12. The labeled monoclonal antibody of Claim 11, wherein said enzyme is alkaline phosphatase, glucose oxidase, galactosidase, or peroxidase.

13. The labeled monoclonal antibody of Claim 10, wherein said label is a fluorescent compound or probe capable of being used in an immuno-fluorescent or fluorescent immunoassay procedure, enzyme fluorescent immunoassay, or fluorescence polarization immunoassay, photon counting immunoassay, or the like procedure.

14. The labeled monoclonal antibody of Claim 13, wherein said fluorescent compound or probe is fluorescein.

15. The labeled monoclonal antibody of Claim 10, wherein said label is a chemiluminescent compound capable of being used in a luminescent or enzyme-linked luminescent immunoassay.

16. The labeled monoclonal antibody of Claim 15, wherein such chemiluminescent compound is luminol or a luminol derivative.

17. The labeled monoclonal antibody of Claim 10, wherein said label is a bioluminescent compound capable of being used in an appropriate bioluminescent immunoassay.

18. The labeled monoclonal antibody of Claim 17, wherein such bioluminescent compound is luciferase or a luciferase derivative.

19. A process for diagnosing for the presence of an antigen of Chlamydia in a specimen comprising contacting at least a portion of said specimen with a labeled monoclonal antibody of Claim 9 in an immunoassay procedure appropriate for said label.

20. The process of Claim 19, wherein the appropriately labeled immunoassay procedure is selected from immuno-fluorescent or fluorescent immunoassay, immuno-electron microscopy, radiometric assay systems, enzyme-linked immunoassays, fluorescence polarization, photon-counting bioluminescent, or chemiluminescent immunoassay.

21. The process of Claim 20, wherein said label is an enzyme capable of being used in an enzyme-linked immunoassay procedure.

22. The process of Claim 21, wherein said enzyme is selected from alkaline phosphatase, glucose oxidase, galactosidase, or peroxidase.

23. The process of Claim 20, wherein said label is a fluorescent compound or probe capable of being used in an immuno-fluorescent or fluorescent immunoassay procedure, enzyme fluorescent immunoassay, or fluorescence polarization immunoassay, or photon-counting immunoassay, or the like procedure.

24. The process of Claim 23, wherein said fluorescent compound or probe is fluorescein.

25. The process of Claim 20, wherein said label is a chemiluminescent compound capable of being used in a luminescent or enzyme-linked luminescent immunoassay.

26. The process of Claim 25, wherein said chemiluminescent compound is luminol or a luminol derivative.

27. The process of Claim 20, wherein said label is a bioluminescent compound capable of being used in a bioluminescent or enzyme-linked bioluminescent immunoassay.

28. The process of Claim 27, wherein said bioluminescent compound is luciferase or a luciferase derivative.

29. A therapeutic composition comprising one or more of the monoclonal antibodies in Claims 1-8 and a pharmaceutically acceptable carrier or diluent.

30. A therapeutic composition comprising one or more of the labeled monoclonal antibodies in Claim 9 and a pharmaceutically acceptable carrier or diluent.

31. A method of treating Chlamydia infections comprising administering an effective amount of a monoclonal antibody of Claims 1-8.

32. A kit for diagnosing for the presence of an antigen or species of Chlamydia in a diagnostic specimen comprising at least one monoclonal antibody of Claims 1-8.

33. The kit of Claim 32, wherein said at least one antibody is labeled.

34. The kit of Claim 33, wherein said at least one monoclonal antibody is labeled with a fluorescent compound.

35. The kit as in Claim 33, wherein said at least one monoclonal antibody is labeled with an enzyme.

36. The kit as in Claim 33, wherein said at least one monoclonal antibody is labeled with a member of the group consisting of a radioactive isotope, chemiluminescent compound, bioluminescent compound, ferromagnetic atom, or particle.

37. The kit of Claims 33, 34, 35, and 36 additionally containing at least one known Chlamydia antigen as a control.

38. The kit of Claims 33, 34, 35, 36, and 37 containing each known antigen of Chlamydia trachomatis, and/or Chlamydia psittaci.

39. The kit of Claims 33, 34, 35, 36, and 37 containing the antigens of Chlamydia trachomatis.

40. The kit of Claims 33, 34, 35, 36, and 37 containing the antigens of Chlamydia psittaci.

41. A kit for diagnosing for the presence of an antigen or species of Chlamydia in a diagnostic specimen comprising at least one monoclonal antibody of Claims 1-8 and a control.

42. The kit of Claim 41, wherein said at least one antigen is labeled and said control is at least one known antigen of Chlamydia.

43. A kit for diagnosing for the presence of a Chlamydia infection comprising at least one monoclonal antibody of Claims 1-8.

44. The kit of Claim 43, wherein said at least one monoclonal antibody is labeled.

INTERNATIONAL SEARCH REPORT

International Application No PCT/GB 85/00466

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [6]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC [4]: C 07 K 15/00; C 12 P 21/00; G 01 N 33/577; G 01 N 33/569; A 61 K 39/40 // C 12 N 15/00 (C 12 P 21/00; C 12 R 1:91)

II. FIELDS SEARCHED

Minimum Documentation Searched [7]

Classification System	Classification Symbols
IPC [4]	C 12 P; G 01 N; A 61 K

Documentation Searched other than Minimum Documentation to the Extent that such Documents are included in the Fields Searched [8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT [9]

Category [10]	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No. [13]
X	The Journal of Immunology, vol. 128, no. 3, March 1982 (Baltimore, MD, US) R.S. Stephens et al.: "Monoclonal antibodies to Chlamydia trachomatis: antibody specifities and antigen characterization", pages 1083-1089, see page 1083, the abstract; page 1088, left-hand column, lines 17-19; page 1089, left-hand column, lines 35-50	1-8,32,41-43
Y		9-28,33-40,44
	--	
X	The Journal of Immunology, vol. 130, no. 6, June 1983 (Baltimore, M.D., US) A. Rosen et al.: "Human monoclonal antibodies to a genus-specific chlamydial antigen, produced by EBV-transformed B cells", pages 2899-2902, see page 2900, right-hand column, lines 17-21 and page 2902, table I and last paragraph	1-8,32,41-43
Y		9-28,33-40,44
	--	

./.

* Special categories of cited documents: [10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
7th February 1986	28 FEV. 1986
International Searching Authority	**Signature of Authorized Officer**
EUROPEAN PATENT OFFICE	M. VAN MOL

Form PCT/ISA/210 (second sheet) (January 1985)

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category *	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No
X	Clinical Chemistry, vol. 28, no. 7, July 1982 (Washington, D.C., US) E.D. Sevier: "The use of monoclonal antibodies for detection and diagnosis of venereal disease", see page 1535, left-hand column, first abstract	1-6,8-10,13-14,19,20,23,24,33,34,37-44
Y		11,12,15-18,21,22,25-28,35,36
X	EP, A, 0098557 (ORION CORP. LTD.) 18 January 1984 see claims 1,5,7; page 15, line 18 - page 16, line 13; page 4, line 20 - page 5, line 2	1-28,32-44
X	WO, A, 83/03678 (HYBRITECH INC.) 27 October 1983 see page 10, line 20 - page 12, line 18	1-8
Y		9-28,33-44
Y	EP, A, 0059624 (THE REGENTS OF THE UNIVERSITY OF CALIFORNIA) 8 September 1982 see claims 1,12-14,16; page 1, lines 6-11; page 3, lines 18-25; page 18, line 26 - page 19, line 4; page 12, lines 7-10	9-28,33-40,44

FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

V. [X] OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE [1]

This international search report has not been established in respect of certain claims under Article 17(2) (a) for the following reasons:

1. [X] Claim numbers 31 because they relate to subject matter not required to be searched by this Authority, namely:

See Rule 39.1.iv. PCT

Methods for treatment of the human or animal body by surgery or therapy, as well as diagnostic methods;

2. [ ] Claim numbers ....., because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. [ ] Claim numbers....., because they are dependent claims and are not drafted in accordance with the second and third sentences of PCT Rule 6.4(a).

VI. [ ] OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING [2]

This International Searching Authority found multiple inventions in this international application as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims of the international application.

2. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the international application for which fees were paid, specifically claims:

3. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers:

4. [ ] As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additional fee.

Remark on Protest

[ ] The additional search fees were accompanied by applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON

INTERNATIONAL APPLICATION NO. PCT/GB 85/00466 (SA 10969)

This Annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report. The members are as contained in the European Patent Office EDP file on 21/02/86

The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
EP-A- 0098557	18/01/84	JP-A-	59027261	13/02/84
		AU-A-	1649283	05/01/84
WO-A- 8303678	27/10/83	AU-A-	1550783	04/11/83
		EP-A-	0105359	18/04/84
		GB-A-	2128630	02/05/84
EP-A- 0059624	08/09/82	JP-A-	57158725	30/09/82
		US-A-	4427782	24/01/84
		CA-A-	1186223	30/04/85